

COVALENT JOINING OF DNA STRANDS TO RNA STRANDS

CATALYZED BY VACCINIA TOPOISOMERASE

Abstract of the Disclosure

The present invention provides a method of covalently joining a DNA strand to an RNA strand comprising (a) forming a topoisomerase-DNA intermediate by incubating a DNA cleavage substrate comprising a topoisomerase cleavage site with a topoisomerase specific for that site, wherein the topoisomerase-DNA intermediate has one or more 5' single-strand tails; and (b) adding to the topoisomerase-DNA intermediate an acceptor RNA strand complementary to the 5' single-strand tail under conditions permitting a ligation of the covalently bound DNA strand of the topoisomerase-DNA intermediate to the RNA acceptor strand and dissociation of the topoisomerase, thereby covalently joining the DNA strand to the RNA strand. The present invention also provides a method of tagging a 5' end of an RNA molecule. The present invention further provides a DNA-RNA molecule which has been joined in vitro by the use of a topoisomerase. The present invention also provides a method of tagging a 5' end of an mRNA. The present invention provides a method of isolating and cloning full-length gene sequences using capped mRNA after subtraction of non-capped RNA.